implicated as regulators of lens development. To understand the signaling mechanisms downstream of receptor tyrosine kinase (RTK), we deleted both ERK1 and ERK2 in mouse lens using a lens-specific Cre transgenic line. We found that ERK1/2-deficient lens looked normal before embryonic day 15.5 (E15.5). However, after E15.5, the epithelial cells in ERK1/2-deficient lens failed to differentiate into the lens fiber cells. As a result, the epithelial layer expanded and the epithelial cells migrated toward the posterior pole of the lens. At the molecular level, none of the differentiation markers, such as fiber cell specific crystallins and transcription factors, was turned on in the epithelial cells at the posterior region. By birth, the mutant lens had completely lost its tissue polarity with a monolayer of epithelial cells surrounding the entire lens. These results suggest that epithelial-to-fiber differentiation during lens development is dependent on the ERK1/2-signaling activity. In addition to the differentiation defect, cell proliferation in the ERK1/2-deleted lens was also severely inhibited after E15.5 as measured by the expression of cell proliferation markers such as BrdU and Ki67. In summary, we conclude that ERK-signaling activity is essential for lens fiber cell differentiation and is also important for cell proliferation during mouse lens development.

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Program/Abstract # 340
Inv compartment of the primary cilia
Takahiko Yokoyama, Dai Shiba
Department of Anatomy, Division of Anatomy and Developmental Biology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Recent advancements have shown that primary cilia have diverse functions in development, cell signaling and cell proliferation. Primary cilia are a tiny hair like structure extending from the cell surface. Yet, its structure is not simple, it is structurally divided along its vertical axis into the ciliary tip, the shaft, the ciliary necklace, the transitional zone and the basal body. More than 200 proteins are present in this tiny organelle. These proteins are thought to be function in each compartment of the cilia. However, intraciliary localization of these proteins has not been well examined or ignored. Nephronophthisis (NPHP) is the most common genetic cause of childhood end-stage renal failure. To date, nine causative genes have been identified, and all of them are reported to locate cilia/centriole. The *inv* mice develop multiple renal cysts, and are a model for NPHP2. Mouse *Inv* gene encodes 1062 amino acids, and the Inv is localized in primary cilia. Here, we present that the Inv is localized at a distinctive proximal segment of the primary cilium, using GFP-tagged *Inv* constructs and anti-Inv antibody. We name this segment the Inv compartment of the cilium. Further investigation of the Inv shows that 60 amino acids at the C-terminal, that contains ninein homologous sequences, are critical for localization to the Inv compartment. These results reveal that the primary cilium has a distinct molecular compartment in the body of the primary cilium. We are also going to present other NPHP proteins localization in the primary cilia.

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Program/Abstract # 341
Identification and characterization of mutation in tau tubulin kinase 2 affecting cilia formation and hedgehog signaling during development
Sarah C. Goetz, Kathryn V. Anderson
Program in Developmental Biology, Sloan Kettering Institute, New York, NY, USA

Previous work by our lab and others has revealed a surprising requirement for primary cilia in mammalian Hedgehog (Hh) signaling, however the mechanisms by which cilia regulate Hh signaling remain an active area of investigation. The mutant *bartleby* (*bby*) was isolated in a recent genetic screen and found to have a neural patterning phenotype consistent with a severe disruption in Hh signaling. Specifically, the neural tube of *bby* mutants lacks a floorplate as well as V3 interneuron and motor neuron progenitors, while dorsal cell types are expanded. Examination of cilia formation by immunostaining and scanning electron microscopy has revealed that *bby* mutants have very short, sparse cilia. This ciliogenesis defect likely to be the cause of the loss of Hh signaling observed in *bby* embryos. We mapped the *bby* mutation to a 1Mb interval on mouse chromosome 2, and identified a single base change in the coding region of tau tubulin kinase 2 (Ttbk2) that would cause a premature stop codon within the kinase domain. Ttbk2 has been previously reported to phosphorylate the microtubule-associated proteins Tau and Map2 and regulate their ability to bind tubulin. While disruptions of Tau, Map2 and Ttbkk2 are associated with human neurodegenerative disorders, their functions during embryonic development are not well established. Examination of the cellular and biochemical defects in Ttbk2^{bby} embryos should elucidate how microtubule dynamics affect cilia formation and Hh signaling.

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Program/Abstract # 342
Tubby-like 3 (TULP3) and mammalian Sonic Hedgehog signaling
Jonathan T. Eggenschwiler, Ryan X. Norman
Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

The mechanism of mammalian Hedgehog signal transduction has been actively investigated, but our understanding of this process is far from complete. Here we identify Tubby-like protein 3 (TULP3) as a novel antagonist of this pathway in mice. *Tulp3* mutants show neural tube and limb patterning defects indicative of unrestrained Sonic Hedgehog (Shh) pathway activity. Epistasis experiments indicate that disruption of *Tulp3* activates the pathway at a step downstream of Sonic Hedgehog and Smoothened and upstream of Gli2. Genetic and cell biological experiments have pointed to a central role played by primary cilia in mammalian Hedgehog signaling and Tubby family proteins have been implicated in a number of cilia-related processes in diverse species. Thus, we hypothesize that TULP3's role in Shh signaling is intimately related to signaling events within the primary cilium. This hypothesis is supported by the observation that TULP3 protein localizes to the tips of primary cilia and by the finding that activation of the Shh pathway in *Tulp3* mutants is suppressed in a genetic background lacking primary cilia.

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Program/Abstract # 343
The Iguana/DZIP protein controls biogenesis of primary cilia
Shang Yew Tay[a], Xianwen Yu[a], Kangli Noel Wong[a], Sudipto Roy[a,b]
[a]*Institute of Molecular and Cell Biology, Cancer and Developmental Cell Biology Division, Proteos, 61 Biopolis Drive, Singapore 138673, Singapore*
[b]*Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore*

Primary cilia are assembled by almost all vertebrate cells as they exit the cell-cycle. Recent studies have associated primary cilia with

the transduction of important morphogenetic signals. The genetic and cell biological control of ciliogenesis, however, is poorly understood. Here we show that mutation of the zebrafish iguana gene strongly inhibits primary cilia formation. Iguana encodes a zinc finger and coiled-coil containing protein, which we have previously implicated in Hedgehog signaling. We now argue that aberrant Hedgehog signaling in iguana mutants arises from their lack of primary cilia. Consistent with this, we have found that like in mammals, the 7-pass transmembrane protein Smoothened translocates to primary cilia in cells of the zebrafish embryo in response to Hedgehog activity. Despite the obligatory requirement of Iguana for primary ciliogenesis, surprisingly, its loss has a relatively mild effect on the assembly and function of motile cilia. Our findings identify the Iguana protein as a novel and critical component of the primary ciliogenic pathway.

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Program/Abstract # 344

Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells

Philip B. Abitua[a], Tae Joo Park[a], Brian J. Mitchell[b], Chris Kintner[b], John B. Wallingford[a]
[a]*Department of Molecular Cell and Developmental Biology, University of Texas, Austin, TX, USA*
[b]*The Salk Institute for Biological Studies, La Jolla, CA, USA*

The planar cell polarity (PCP) signaling system governs many aspects of polarized cell behavior. Here, we use an *in vivo* model of vertebrate mucociliary epithelial development to show that Dishevelled (Dvl) is essential for the apical positioning of basal bodies. We find that Dvl and Inturned mediate the activation of the Rho GTPase specifically at basal bodies, and that these three proteins together mediate the docking of basal bodies to the apical plasma membrane. Moreover, we find that this docking involves a Dvl-dependent association of basal bodies with membrane-bound vesicles and the vesicle-trafficking protein, Sec8. Once docked, basal bodies again require Dvl and Rho for the planar polarization that underlies directional beating of cilia. These results demonstrate previously undescribed functions for PCP signaling components and suggest that a common signaling apparatus governs both apical docking and planar polarization of basal bodies.

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Program/Abstract # 345

PCP signaling: A genome-wide screen for new Rho kinase substrates

Gretchen L. Dollar[a], Cathie Pfleger[b], Andreas Jenny[a]
[a]*Department of Mol. and Dev. Biology, Albert Einstein College of Medicine, Bronx NY, USA*
[b]*Department of Oncological Sciences, Mt. Sinai School of Medicine, NY, USA*

Planar Cell Polarity (PCP) signaling regulates the establishment of polarity within the plane of a tissue, and is required for the determination of cell fates, the generation of asymmetric, but highly aligned structures (e.g. stereocilia in the human inner ear or fly wing hairs), and the directional migration of cells during convergent extension, a process required for vertebrate gastrulation and neural tube closure. PCP is governed by the non-canonical Fz/PCP pathway, in which activation of a Fz receptor leads to nuclear responses, as well as to cytoskeletal changes mediated by Rho Kinase (Drok). In *Drosophila*, PCP is essential for the alignment of ommatidia in the eye, which requires proper specification of photoreceptor cells, as well as the coordinated movement of groups of photoreceptor cells, thus making it an ideal system to analyze PCP signaling *in vivo*. We performed a genome wide *in vitro* screen to identify new Drok substrates using a phosphorylation induced gel-shift assay. We are currently characterizing candidates using *in vivo* RNAi, mutational analysis, and genetic interaction assays with known PCP and Drok pathway components. One new Rho Kinase substrate we identified is the formin *frl*. Formins are known to regulate actin polymerization dynamics, and the *Xenopus* formin XDAAM was previously shown to be activated by Dishevelled during convergent extension. *Frl* genetically interacts with Drok, and its knock-down causes PCP phenotypes in the eye, suggesting *frl* may be the first *Drosophila* formin identified that is required for PCP signaling.

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Program/Abstract # 346

Distinct developmental roles of planar cell polarity proteins vangl1, prickle1, and prickle2 in cortical crescents and primary cilia

Dragana Antic[a,b], Kaye Suyama[b], Jeffrey D. Axelrod[a], Matthew P. Scott[b]
[a]*Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA*
[b]*Departments of Developmental Biology, Genetics, and Bioengineering, HHMI, Stanford University School of Medicine, Stanford, CA, USA*

Planar cell polarity (PCP) is manifested as the coordinated, polarized orientation of cells within epithelial sheets, or as directional cell migration and intercalation during convergent extension. Several PCP genes regulate these two developmental processes but their individual roles and interactions are poorly understood. Ciliary mutants display PCP defects, revealing that primary cilia also play a role in PCP. Genetic interaction between Vangl2 and Vangl1 has been observed, but the specific function of Vangl1 in PCP remains uncharacterized. Here, we show that mouse Vangl1 regulates leftright asymmetry in the ventral node, and that it cooperates with Vangl2 to regulate PCP in the vestibular epithelium. In both tissues, VANGL1 protein co-localizes with other PCP proteins in two different subcellular compartments: in apical cortical crescents, a conserved pattern typical of PCP proteins, as well as in a subset of primary cilia. These results confirm that some aspects of PCP signaling are highly conserved from flies to mammals. The presence of PCP proteins in primary cilia suggests a unique vertebrate-specific aspect of PCP signaling, which may explain the requirement for primary cilia in planar polarity.

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